

Application No. 10/758,237

AMENDMENTS TO THE CLAIMS

A detailed listing of all claims that are, or were, in the present application, irrespective of whether the claim(s) remains under examination in the application are presented below. The claims are presented in ascending order and each includes one status identifier.

1. (Original) A transposon comprising
a transcriptional unit and a plurality of insulator elements, wherein the transcriptional unit is flanked by at least one insulator element on each side of the transcriptional unit, wherein the transcriptional unit comprises an exogenous nucleic acid for introduction into a cell.
2. (Original) The transposon of claim 1, wherein the transposon comprises at least two inverted repeat sequences.
3. (Original) The transposon of claim 1, wherein the insulator element specifically binds to a CTCF protein.
4. (Original) The transposon of claim 1, wherein the insulator element comprises a binding site for a CTCF protein.
5. (Original) The transposon of claim 1, wherein the insulator element comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:16.

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6. (Original) The transposon of claim 1, wherein the insulator element comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:17.
7. (Original) The transposon of claim 1, wherein the insulator element comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:18.
8. (Original) The transposon of claim 1, wherein the insulator element comprises at least one of SEQ ID NO: 16, SEQ ID NO:17, OR SEQ ID NO:18.
9. (Original) The transposon of claim 1, wherein at least one of the insulator elements comprises a *scs/scs'* element from the *D. melanogaster* 87A7 heat shock locus.
10. (Original) The transposon of claim 1, wherein at least one of the insulator elements comprises a gypsy moth retrotransposon *su(hW)* binding region-type insulator.
11. (Original) The transposon of claim 1, wherein at least one of the insulator elements comprises an A-element-type insulator.
12. (Original) The transposon of claim 1, wherein at least one of the insulator elements comprises an A-element-type insulator from a chicken lysozyme locus.

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13. (Original) The transposon of claim 1, wherein at least one of the insulator elements comprises an insulator element from a DHS5 site of a chicken α -globulin locus.
14. (Original) The transposon of claim 1 wherein at least one of the insulator elements comprises the mammalian *apolipoprotein B* gene insulator.
15. (Original) The transposon of claim 1 wherein at least one of the insulator elements comprises a human α -interferon gene insulator.
16. (Original) The transposon of claim 1 wherein at least one of the insulator elements comprises an *sns* insulator from sea urchin arylsulfatase gene.
17. (Original) The transposon of claim 1 wherein at least one of the insulator elements comprises a *Drosophila bithorax Fab-7* insulator.
18. (Original) The transposon of claim 1 wherein at least one of the insulator elements comprises a mammalian insulator flanking a tyrosinase gene.
19. (Original) The transposon of claim 1, wherein the transcriptional unit is disposed between a first insulator element and a second insulator element, and the first insulator element and the second insulator element are disposed between inverted repeats of a transposon.

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20. (Original) The transposon of claim 1, wherein the transcriptional unit further comprises at least one member of the group consisting of promoters and enhancers.
21. (Original) The transposon of claim 1 wherein the exogenous nucleic acid encodes a marker molecule.
22. (Original) The transposon of claim 1 wherein the exogenous nucleic acid is a member of the group consisting of DNA encoding an antisense RNA or siRNA.
23. (Original) The transposon of claim 1 wherein the exogenous nucleic acid is a member of the group consisting of DNA encoding an mRNA.
24. (Original) The transposon of claim 1 comprising at least two inverted repeat sequences that specifically bind to a Sleeping Beauty transposase.
25. (Original) The transposon of claim 1 further comprising a suicide sequence nucleic acid.
26. (Original) The transposon of claim 13 further comprising an independent promoter for the suicide sequence nucleic acid.
27. (Original) A cell, the cell comprising the transposon of claim 1.

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28. (Original) The cell of claim 27, wherein the cell is in vitro.
29. (Original) The cell of claim 27, wherein the cell is in an animal.
30. (Original) The cell of claim 27, wherein the cell is in a human.
31. (Original) The cell of claim 27, wherein the cell is transfected with the transposon.
32. (Original) The cell of claim 27, wherein the cell is electroporated with the transposon.
33. (Original) The cell of claim 27, wherein the cell is microinjected with the transposon.
34. (Original) The cell of claim 27, wherein the cell is electroporated or microinjected with the transposon, and the cell produces a protein that is encoded by the exogenous nucleic acid.
35. (Original) The cell of claim 27, wherein the cell is transfected with the exogenous nucleic acid and produces a protein that is encoded by the exogenous nucleic acid.
36. (Original) The cell of claim 27, wherein the cell is a member of the group consisting of lymphocytes, pancreatic cells, neural cells, muscle cells, and blood cells.

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37. (Original) The cell of claim 27, wherein the cell is a member of the group consisting of hepatocytes, hepatoma cells, primary hepatocytes and liver cells.
38. (Original) The cell of claim 27 wherein the cell wherein the cell is a stem cell.
39. (Original) The cell of claim 27, wherein the cell wherein the cell is a member of the group consisting of primary pancreatic cells and pancreatic stem cells.
40. (Original) The cell of claim 27, wherein the cell wherein the cell is a member of the group consisting of primary hematopoietic cells and hematopoietic stem cells.
41. (Original) The cell of claim 32, wherein the protein is a marker.
42. (Original) The cell of claim 32, wherein the protein is a therapeutic protein.
43. (Original) The cell of claim 34, wherein the therapeutic protein ameliorates a medical condition.
44. (Original) An animal, the animal comprising the transposon of claim 1.
45. (Original) The animal of claim 44 wherein the animal is a member of the group consisting of a zebrafish, a mouse, and a rat.

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46. (Original) An animal embryo, the embryo comprising the transposon of claim 1.
47. (Original) The animal embryo of claim 46 wherein the embryo is a member of the group consisting of a zebrafish, a mouse, and a rat.
48. (Original) A method of altering a cell, the method comprising exposing the cell to a transposon that comprises a transcriptional unit and a plurality of insulator elements, wherein the transcriptional unit is flanked by at least one insulator element on each side of the transcriptional unit, wherein the transcriptional unit comprises an exogenous nucleic acid for introduction into a cell.
49. (Original) The method of claim 48 wherein the transposon is introduced into the cell by electroporation.
50. (Original) The method of claim 48 wherein the transposon is introduced into the cell by microinjection.
51. (Original) The method of claim 48 wherein the cell is a member of the group consisting of lymphocytes, pancreatic cells, neural cells, muscle cells, and blood cells.
52. (Original) The method of claim 48 wherein the cell is a member of the group consisting of hepatocytes, hepatoma cells, primary hepatocytes and liver cells.

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53. (Original) The method of claim 48 wherein the cell is a stem cell.
54. (Original) The method of claim 48 wherein the cell is a member of the group consisting of primary pancreatic cells and pancreatic stem cells.
55. (Original) The method of claim 48 wherein the cell is a member of the group consisting of primary hematopoietic cells and hematopoietic stem cells.
56. (Original) The method of claim 48, wherein the insulator element specifically binds to a CTCF protein.
57. (Original) The method of claim 48, wherein the insulator element comprises a binding site for a CTCF protein.
58. (Original) The method of claim 48, wherein the insulator element comprises a 16-base sequence that has at least 80% identity with SEQ ID NO: 16.
59. (Original) The method of claim 48, wherein the insulator element comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:17.
60. (Original) The method of claim 48, wherein the insulator element comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:18.

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61. (Original) The method of claim 48, wherein the insulator element comprises at least one member of the group consisting of SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18.

62. (Original) The method of claim 48, wherein at least one of the insulator elements is chosen from a member of the group consisting of *scs/scs'* elements from the *D. melanogaster* 87A7 heat shock locus, a gypsy moth retrotransposon *su(hW)*-binding region, an A-element, an A-element from a chicken lysozyme locus, a DHS5 site of a chicken α -globulin locus, a mammalian *apolipoprotein B* gene insulator, a human α -interferon gene insulator, an *sns* insulator from sea urchin arylsulphatase gene, a *Drosophila bithorax Fab-7* insulator, and a mammalian insulator flanking a tyrosinase gene.

63. (Original) The method of claim 48, wherein the transcriptional unit is disposed between a first insulator element and a second insulator element, and the first insulator element and the second insulator element are disposed between at least two inverted repeats.

64. (Original) The method of claim 48, wherein the transcriptional unit further comprises at least one member of the group consisting of promoters and enhancers.

65. (Original) The method of claim 48 wherein the exogenous nucleic acid encodes a marker molecule.

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66. (Original) The method of claim 48 wherein the exogenous nucleic acid is a member of the group consisting of antisense DNA, DNA, and cDNA.
67. (Original) The method of claim 48 wherein the exogenous nucleic acid encodes siRNA.
68. (Original) The method of claim 48 comprising at least two inverted repeat sequences that specifically bind to a Sleeping Beauty transposase.
69. (Original) The method of claim 48 further comprising a suicide sequence nucleic acid.
70. (Original) The method of claim 69 further comprising an independent promoter for the suicide sequence nucleic acid.
71. (Original) The method of claim 48 further comprising exposing a cell to the transposon.
72. (Original) The method of claim 71, wherein the cell is in vitro.
73. (Original) The method of claim 71, wherein the cell is in an animal.
74. (Original) The method of claim 71, wherein the cell is in a human.

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75. (Original) The method of claim 71, wherein the cell is transfected with the exogenous nucleic acid.
76. (Original) The method of claim 71, wherein the cell is electroporated or microinjected with the transposon.
77. (Original) The method of claim 71, wherein the cell is transfected with the exogenous nucleic acid and produces a protein that is encoded by the exogenous nucleic acid.
78. (Original) The method of claim 77, wherein the protein is a marker.
79. (Original) The method of claim 77, wherein the protein is a therapeutic protein.
80. (Original) The method of claim 79, wherein the therapeutic protein ameliorates a medical condition.
81. (Original) The method of claim 48 further comprising exposing a cell in an animal to the transposon.
82. (Original) The method of claim 81 wherein the animal is a member of the group consisting of a zebrafish, a mouse, and a rat.

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83. (Original) The method of claim 81 wherein the animal is an embryo.
84. (Original) The method of claim 83 wherein the embryo is a member of the group consisting of a zebrafish, a mouse, and a rat.
85. (Currently Amended) A transposon comprising a transcriptional unit and a means for preventing regulation of transcription of host nucleic acid by the transcriptional unit following insertion [[of]] into a host mammalian cell ~~nuclear genome~~.
86. (Original) The transposon of claim 85 wherein the host nucleic acid is a gene.
87. (Original) The transposon of claim 85 wherein the transcriptional unit comprises an exogenous nucleic acid.
88. (Original) The transposon of claim 85, wherein the means for preventing regulation of transcription of host nucleic acid specifically binds to a CTCF protein.
89. (Original) The transposon of claim 85, wherein the means for preventing regulation of transcription of host nucleic acid comprises a binding site for a CTCF protein.

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90. (Original) The transposon of claim 85, wherein the transcriptional unit is disposed between a first insulator element and a second insulator element, and the first insulator element and the second insulator element are disposed between the at least two inverted repeats.

91. (Original) The transposon of claim 85, wherein the transcriptional unit further comprises at least one member of the group consisting of promoters and enhancers.

92. (Original) The transposon of claim 85 wherein the transcriptional unit encodes a marker molecule.

93. (Original) A cell, the cell comprising the transposon of claim 85.

94. (Original) The cell of claim 93, wherein the cell is in vitro.

95. (Original) The cell of claim 94, wherein the cell is in a human.

96. (Original) An animal, the animal comprising the transposon of claim 85.

97. (Original) The animal embryo of claim 96 wherein the embryo is a member of the group consisting of a zebrafish, a mouse, and a rat.

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98. (Original) The transposon of claim 85, wherein the means for preventing regulation of transcription of host nucleic acid comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:16

99. (Original) The transposon of claim 85, wherein the means for preventing regulation of transcription of host nucleic acid comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:17

100. (Original) The transposon of claim 85, wherein the means for preventing regulation of transcription of host nucleic acid comprises a 16-base sequence that has at least 80% identity with SEQ ID NO:18.

101. (Original) The transposon of claim 85, wherein the means for preventing regulation of transcription of host nucleic acid comprises at least one member of the group consisting of SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18 .

102. (Original) The transposon of claim 85 wherein the transcriptional unit is a member of the group consisting of antisense DNA, DNA, and cDNA.